

**3030-Pos Board B185****Differential Effects of Liquid Ordered and Liquid Disordered Phases on Membrane Permeability**

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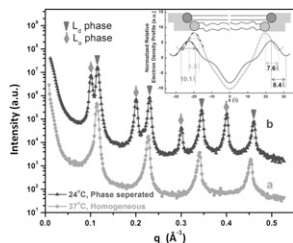
Phospholipid membranes segregate into lateral domains of liquid ordered (lo) and liquid disordered (ld) phases when cholesterol and mixed species of lipids with saturated and unsaturated acyl chains are present. To examine membrane permeability and detergent solubility in pure lo, pure ld, and mixed lo/ld phases, LUVs were prepared based on the ternary phase diagram of POPC, sphingomyelin, and cholesterol. These LUVs were loaded with 2mM carboxyfluorescein (CF) and formed by extrusion at 70°C. using a stopped-flow fluorometer, changes in CF fluorescence were measured when LUVs were exposed to sudden osmotic gradients, pH gradients, or 0.1% Triton. Acyl chain and phospholipid headgroup packing were assessed in all compositions with time-resolved measurements of DPH fluorescence lifetime and anisotropy decay. Water permeability was highest in the pure ld phase, and a factor of more than 100 lower in the pure lo phase. In the lo/ld coexistence region water permeability decreased approximately exponentially with increasing percent lo phase. Proton permeability was lowest in the pure ld phase, increasing linearly with increasing percent lo up to the "percolation point" (connected phase switches from lo to ld) at which point it remained approximately the same with increased lo. The rate of membrane solubilization was highest in the pure ld phase and did not decrease substantially until the percolation point was reached, and decreased by a factor of 40 in the pure lo phase. Water permeability was found to correlate approximately exponentially with acyl chain packing, decreasing with increased membrane order. Proton permeability increased linearly with increasing membrane order.

**3031-Pos Board B186****Long-Range Smectic Coupling of Phase Separated Domains in Ternary Mixture Lipid Multilayers**

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Lipid multilayers serve as suitable and convenient bio-mimetic systems and are broadly used for studies of lipid membrane structure and function. It is known that many mixed lipid systems undergo phase separation as a function of temperature. Here we report that, in multilayers, the lateral phase separation in the bilayers is accompanied by long-range columnar order of the two phases along the normal to the bilayers arising from the coupling of two-dimensional intra-layer phase separation and inter-layer smectic ordering. Quantitative analysis of real-time dynamical experiments of confocal fluorescence microscopy reveal an interplay between intra-layer domain growth and inter-layer coupling, while X-ray reflectivity studies establish that the phase-separated domains are correlated normal to the lamellae over hundreds of bilayers. Through reconstruction of relative electron density profiles, XRR data also offer insight into differences in the domain structure on nm length scales. The microscopic understanding of two coexisting and domain-aligned multilamellar phases advanced by our experiments shed new light on the role of water in organizing membrane phases in stacked bilayers - a phenomenon of possible relevance to the mechanism of inter-layer lipid-lipid interactions in biological membranes.

**3032-Pos Board B187****Deuterium NMR Study of the Effect of Phytosterol on DPPC Membranes**

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We investigate the phase behavior of model membranes composed of 1, 2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and phytosterol using deuterium nuclear magnetic resonance (NMR). The *sn*-1 chain of DPPC is deuterium labeled. The deuterium NMR spectra were taken as a function of temperature and phytosterol concentration. Our data shows that phytosterol

promotes the formation of the liquid-ordered phase. Moreover, this phytosterol has opposite ordering effect on the DPPC membranes below and above  $T_m$ : It decreases the order of DPPC membranes below  $T_m$ , whereas increases the chain orders of DPPC above  $T_m$ . In addition, the partial phase diagram is determined. There are two two-phase coexistence regions found below and above  $T_m$  at intermediate sterol concentration.

**3033-Pos Board B188****Dependence of Cholesterol-Phospholipid Affinity on Degree of Acyl Chain Unsaturation as Determined by EPR**

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Cholesterol-lipid interactions are thought to play an intrinsic role in determining lateral organization within cellular membranes. Cholesterol's tendency to restrict acyl chain motion makes interaction with highly disordered polyunsaturated phospholipid less energetically favorable compared with its interactions with more saturated species. The high affinity that the sterol has for saturated chains is the "glue" that holds cholesterol and sphingomyelin rich lipid rafts together. Conversely, poor affinity for cholesterol is hypothesized to drive the formation of polyunsaturated fatty acid (PUFA)-containing phospholipid domains depleted in the sterol. Here we describe a novel method using electron paramagnetic resonance (EPR) to measure the affinity of cholesterol for phospholipids with varying degrees of acyl chain unsaturation. We monitor the partitioning of the spin labeled cholesterol analog, cholestane, between large unilamellar vesicles (LUV) and cyclodextrin (cyd) through analysis of EPR spectra. Because the EPR spectrum of cholestane is sensitive to the very different tumbling rates in the two environments, the ratio of the two populations can be determined by deconvolution of the spectra. Our data show that a decreased affinity of cholesterol accompanies increasing acyl chain unsaturation. The virtue of this EPR method is that it provides a qualitative measure of cholesterol binding that is quick and avoids the necessity for physical separation of LUV and cyd.

**3034-Pos Board B189****Disordering of Raft Domains by DHA and EPA observed with Solid-State Deuterium NMR Spectroscopy**

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Research continues to examine the health benefits of omega-3 polyunsaturated fatty acids (n-3 PUFA) found in fish oils. The major bioactive components are eicosapentaenoic acid (EPA, 20:5), with 20 carbons and 5 double bonds, and docosahexaenoic acid (DHA, 22:6), with 22 carbons and 6 double bonds. However, their molecular modes of action remain unclear. A suggested hypothesis is that these fatty acids are incorporated into membrane phospholipids and modify the structure and organization of lipid rafts, thus affecting cell signaling. We used solid-state <sup>2</sup>H NMR spectroscopy to compare molecular organization in mixtures of 1-palmitoyl-2-eicosapentaenoylphosphatidylcholine (PEPC) and 1-palmitoyl-2-docosahexaenoylphosphatidylcholine (PDPC) with the raft-stabilizing molecules sphingomyelin (SM) and cholesterol. Our spectra for PEPC-d<sub>31</sub> and PDPC-d<sub>31</sub>, analogs of PEPC and PDPC with a perdeuterated palmitoyl *sn*-1 chain, showed that DHA has a greater tendency than EPA to incorporate into raft-like domains enriched in SM and cholesterol. By using PSM-d<sub>31</sub>, an analog of SM with a perdeuterated *N*-palmitoyl chain, we now directly observe one of the raft-forming molecules and analyze the effects EPA and DHA have on molecular order within the raft. These results will add to the growing information on how EPA and DHA differentially modify lipid domain organization in bilayers.

**3035-Pos Board B190****Area Per Lipid and Elastic Deformation of Membrane Bilayers under Osmotic Stress**

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